

REMARKS

Claim 13 has been deleted and various typographical errors noted by the examiner have been corrected. In claim 14, the term “bulkiness” has been retained. Applicant’s attorney is advised that this term is well known to those skilled in the art as meaning the mass of the crude extract rather than its volume.

The Examiner takes the view that claims 12 and 15 - 19 should be withdrawn as being distinct from claim 1. It is submitted that this view is incorrect. All of these claims are dependent on claim 1 and hence include all the limitations of that claim. (35 USC 112 paragraph 4). They cannot therefore be distinct from it or define a different invention. Claim 12 relates to the means of selecting material for use in the present invention. (See Example 2) Claims 15-17 are directed to carrying out Step (a) by use of an ethylene dichloride/dichloromethane solvent, claim 15 using fresh fruit and a pulp:solvent ratio of 1:2, claim 16 using dry fruit and a pulp:solvent ratio of 1:3 and claim 17 using fresh fruit and a pulp:solvent ratio of 1:6. All are described in Example 2. Claim 18 is directed to carrying out Steps (a), (b) and (c) by forming a methanolic extract in Step (a) concentrating this in Step (b) and then extracting imperatorin with an ethylene dichloride/dichloromethane extract and claim 19 is directed to carrying out Steps (a), (b) and (c) by forming a methanolic extract in Step (a), concentrating this in Step (b) and then extracting imperatorin with dichloromethane in Step c). The claims have been amended to clarify their relation to the steps set out in claim 1.

Turning now to the rejection of claims 1.-5, '7 and 9 - 11, 14 and 19 under 35 USC 103 over Saha *et al*, in view of Bastnet *et al*, and Bizhartova *et al*, the present invention provides a step-wise process for isolation of imperatorin, a potent inducible nitric oxide synthase inhibitor and antiinflammatory candidate, from fresh/dried powdered pulps of fruits of *Aegle marmelos* Correa .

As explained in the present application, furanocoumarins such as imperatorin are often found together with other compounds from which their separation is difficult. Use of polar solvents for extraction results in extracts having a high amount of color and fatty material that must be removed to produce a pure product.

The present invention requires extraction of fresh/dried powdered pulp of fruits with a halogenated solvent (dichloromethane, chloroform, carbon tetrachloride, ethylenedichloride) directly or by a first extraction with a monohydric alcohol and then concentrating the extract and partitioning the imperatorin into a halogenated solvent. Such alcoholic extraction may be with methanol or ethanol for 24-48 hours in a ratio of 1:3 to 1:6 pulp to solvent or in Soxhlet apparatus for 6 to 12. hours. The alcoholic extract is concentrated to 10--30 % of its original volume under vacuum. The concentrate is then partitioned with a halogenated solvent in order to transfer the imperatorin to non-polar solvent. The resultant concentrate is then evaporated over anhydrous sodium sulphate, crystallized and filtered. The filtrate is then subjected to vacuum liquid chromatography on silica gel and imperatorin eluted in a solvent to obtain a phytosterol enriched fraction and pure imperatorin fraction. Use of the present invention gives yields of 3.1%, 0.89% and 1.71% for mature, immature and ripe fruits respectively.

Further the present invention teaches efficient and economical extraction of imperatorin with high purity (85%) and 90% (Example 3) and better yield of product as compared to prior art. Additionally, immunologically important phytosterol mixture-enriched fractions were obtained as useful by-products of the present invention (0.04 to 0.1.6% (Example 3) and (0.10% (Example 4). Use of the halogenated solvent in the present invention either directly or to partition an alcoholic extract avoids the problems associated with use of benzene as discussed in the present application and also improvement over the use of polar solvents as discussed above.

Turning now to the art cited by the examiner, Saha *et al.* teaches isolation of imperatorin from the mother liquor left after separation of allo-imperatorin. Fruit pulp was crushed in a Warring blender in a alcohol medium and kept for a month. The alcoholic mother liquor was chromatographed over a column of Brockmann alumina and eluted with benzene. The first four fractions on three crystallizations from a mixture with petroleum ether and benzene (1.:4) furnished colorless fine needle shaped crystals of imperatorin, a yield of 0.006%. Isolation of imperatorin occurs as a step in the isolation of β -sitosterol. The process is not only time consuming but also expensive and results in an extremely poor yield.

Basnet *et al* teaches screening of 33 herbal medicines for anti-itching activities, especially methanol extracts of fruits of *Cnidium monnieri* (Cnidii Fructus). The chloroform soluble fraction of the methanol extract mainly contained xanthotoxin, isopimpinellin, bergapten, imperatorin and osthol and not pure imperatorin.

Bizhanova *et al.* teaches isolation of coumarins by alcoholic. extraction and adsorption chromatography on a column with silica gel. The extract consisted of 6 compounds; 3 furocoumarins identified as pranchimgin, imperatorin and saxalin while the water soluble fraction contained 2 furocoumarins, identified as oxypeucedanin and oxypeucedanin hydrate, one compound was unidentified. Here again imperatorin was not isolated alone but with other compounds.


The present invention's selective extraction into a halogenated solvent or transfer of imperatorin from an alcoholic extract to a halogenated non- polar phase results in easy purification and isolation of pure imperatorin. In contrast: to the cited prior art, imperatorin is isolated both from the crude extract as well as by vacuum chromatography resulting in higher yield and purity. The present invention therefore demonstrates substantial advantages over the cited prior art and is in no way taught by the art.

It is therefore submitted that the present invention provides advantages that are in no way foreshadowed in the art when read separately or in combination. Furthermore there is no other reason why one skilled in the art would have read the references in combination.

It is therefore submitted that the requirements of 35 USC 103 have been met.

In view of the foregoing, it is submitted that this application is now in order for allowance and an early action to this end is respectfully solicited.

Respectfully submitted,



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